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BI 3ZOMY Kit

For In Vitro Diagnostic Use

Cat. No. 20-900-50

Kit Components

The Kit includes reagents for 50 tests (2 x 25) using the 5-Chromosome PCR Mix, and 25 (1 x 25) additional tests each for Chromosome 21 PCR Mix and Sex Chromosome PCR Mix.

5-Chromosome PCR Mix	(Yellow Cap)	20-900-50 part 1	(X2) 500µl
Chromosome 21 PCR Mix	(White Cap)	20-900-50 part 2	(X1) 500µl
Sex Chromosome PCR Mix	(Red Cap)	20-900-50 part 3	(X1) 500µl
PCR Enzyme	(Orange Cap)	20-900-50 part 4	(X4) 25µl

Read More ↓

E59/5 05/14

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1. Product Description and Principles of the Procedure

The 3ZOMY Kit is a DNA-based multiplex QF-PCR (Quantitative Fluorescent Polymerase Chain Reaction) assay designed to test aneuploidy of 5 chromosomes (13, 18, 21, X, and Y) in DNA. DNA can be extracted from prenatal samples: amniotic fluid (AF), chorionic villus (CVS) and fetal tissue samples to determine fetal aneuploidies. A parent (mother or father) sample (blood or tissue) is used as a control for ensuring the origin of the fetal sample and to prevent sample mixing. The product is not intended nor tested to be used for non invasive procedures.

The 3ZOMY Kit includes a multiplex STR marker set used to perform initial aneuploidy diagnosis of the chromosomes 21, 18, 13, X, and Y, and two additional chromosome-specific marker sets for the chromosomes 21 and XY that can be used to confirm the diagnosis.

The 3ZOMY Kit uses a five-dye fluorescence system and is intended for DNA fragment analysis on Applied Bio systems Genetic Analyzers (ABI 310, 3100, 3130, 3500, and 3730).

The 3ZOMY Kit is for professional and special trained users.

QF-PCR analysis includes amplification, detection, and analysis of chromosome-specific, repeated DNA sequences known as short tandem repeat (STR).Fluorescently labeled primers are used for amplification, visualization, and quantification of the fluorescently labeled PCR products.

The resulting PCR products are separated and analyzed by a genetic analyzer. DNA amplified from a normal diploid sample will present two alleles of a chromosome-specific STR marker as two peaks in a 1:1 ratio when the marker is heterozygous (Fig.1) or as one peak when the marker is homozygous (Fig.2).The relative amount of the PCR product is quantified by calculating the allele peaks' height ratio or areas.

STRs vary in size between subjects, depending on the number of tetra repeats present on each allele. Heterozygous STR markers are considered to be informative.

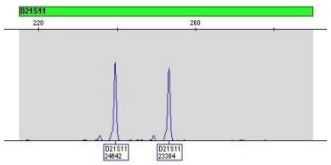


Figure 1.Heterozygous marker (area ratio 1:1)

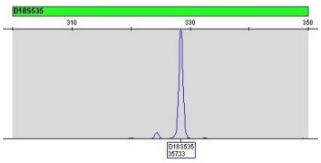


Figure 2. Homozygous/monosomic marker

The detection of three peaks in a 1:1:1 ratio (Fig.3), or two peaks in a 2:1/1:2 ratio (Fig.4), may indicate the presence of an additional STR sequence possibly corresponding to an additional chromosome, as in the case of trisomy.

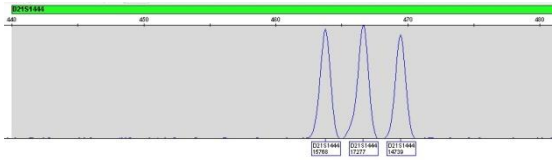


Figure 3.Trisomic marker (area ratio 1:1:1)

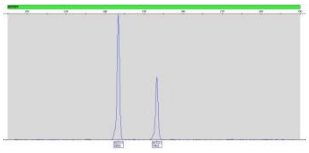


Figure 4. Trisomic marker (area ratio 1:2)

3ZOMY Chromosome Marker Overview

	Genetic Marker	Location	Size Range (bp)	Dye Color
Chromosome 13	D13S742	13q12.12	222 - 334	Green
	D13S634	13q21.32-q21.33	365 - 435	Blue
	D13S628	13q31.1	420 - 475	Yellow
	D13S305	13q13.3	435 - 505	Green
	D13S1492	13q21.1	100 - 175	Red
Chromosome 18	D18S978	18q12.3	195 - 230	Yellow
	D18S535	18q12.3	300 - 350	Blue
	D18S386	18q22.1	338 - 430	Green
	D18S976	18p11.31	440 - 495	Red
	GATA178F11	18p11.32	350 - 410	Yellow
Chromosome 21	D21S1435	21q21.3	150 - 208	Blue
	D21S11	21q21.1	215 - 290	Blue
	D21S1411	21q22.3	245-345	Yellow
	D21S1444	21q22.13	440-495	Blue
	D21S1442	21q21.3	362-420	Red
	D21S1437	21q21.1	105-152	Yellow
Chromosomes X and Y	AMELX/AMELY	Xp22.2,Yp11.2	X=104, Y=110	Blue
	DXYS267	Xq21.31, Yp11.31	175-217	Green
	DXYS218	Xp22.33, Yp11.32	215-260	Red
	DXS1187	Xq26.2	120-170	Green
	XHPRT	Xq26.2-q26.3	265-308	Red
	DXS2390	Xq27.1-q27.2	312-357	Red
	SRY	Yp11.31	236	Yellow
	ZFY, ZFX	Yp11.31, Xp22.11	157-166	Yellow
	TS3	3p24.2, Xq21.1	3=133, X=137	Blue
	TS7	7q34, Xq13	7=181, X=201	Red

Table 1. 3ZOMY genetic markers for 5-Chromosome PCR Mix

	Genetic Marker	Location	Size Range (bp)	Dye Color
Chromosome 21	D21S1435	21q21.3	150 - 208	Blue
	D21S11	21q21.1	215 - 290	Blue
	D21S1411	21q22.3	245-345	Yellow
	D21S1444	21q22.13	450-505	Yellow
	D21S1442	21q21.3	362-420	Red
	D21S1437	21q21.1	105-152	Yellow
	D21S2055	21q22.2	385-488	Blue
	D21S1409	21q21.2	160-220	Green
	D21S1280	21q22.11	295-470	Green

Table 2. 3ZOMY – genetic markers for Chromosome 21 PCR Mix

	Genetic Marker	Location	Size range (bp)	Dye Color
Sex Chromosomes	AMELX/AMELY	Xp22.2,Yp11.2	X=104, Y=110	Green
	DXYS267	Xq21.31, Yp11.31	175-217	Blue
	DXYS218	Xp22.33, Yp11.32	215-260	Red
	DXS1187	Xq26.2	120-170	Green
	XHPRT	Xq26.2-q26.3	265-308	Yellow
	DXS2390	Xq27.1-q27.2	312-357	Red
	DXS6807	Xp22.33	245-280	Blue
	DXS981	Xq13.1	230-260	Green
	DXS6809	Xq21.33	290-340	Blue
	DXS6854	Xq25-26.1	392-430	Green
	DXS6803	Xq21.31	100-140	Blue
	sY625	Yq11.21	247-253	Green
	SRY	Yp11.31	236	Blue
	ZFY, ZFX	Yp11.31, Xp22.11	157-166	Yellow
	TS2	2p23.2, Xq23	2=118, X=114	Yellow
	TS3	3p24.2, Xq21.1	3=133, X=137	Red
	TS7	7q34, Xq13	7=181, X=201	Green

Table 3. 3ZOMY – genetic markers for Sex Chromosome PCR Mix

2. Product Components and Storage Conditions

2.1 Kit Components

The Kit includes reagents for 50 tests (2 x 25) using the 5-Chromosome PCR Mix, and 25 (1 x 25) additional tests each for Chromosome 21 PCR Mix and Sex Chromosome PCR Mix.

5-Chromosome PCR Mix	(Yellow Cap)	20-900-50 part 1	(X2) 500µl
Chromosome 21 PCR Mix	(White Cap)	20-900-50 part 2	(X1) 500µl
Sex Chromosome PCR Mix	(Red Cap)	20-900-50 part 3	(X1) 500µl
PCR Enzyme	(Orange Cap)	20-900-50 part 4	(X4) 25µl

2.2 Reagents Required But Not Supplied

Reagent Preparation

- Consumables for the thermal cycler.
- Micropipette/dispenser with aerosol barrier tips or displacement tips.
- Disposable protective gloves (powder free).

PCR Amplification

- Thermal cycler: ABI GeneAmp® PCR System 9700, using 9600 mode.
- Micropipette/dispenser with aerosol barrier tips or displacement tips.

Detection

- ABI Genetic Analyzer (ABI 310, 3100, 3130, 3500, 3730).
- Performance optimized polymers: POP-4™ or POP-7™.
- Hi-Di™ Formamide, Genetic Analysis Grade.
- 1x Genetic Analyzer Buffer.
- Micropipette/multipipette/dispenser with aerosol barrier tips or displacement tips.
- Spectral calibration dye set: 5-Color Dye Set (BI Cat# 20-910-00) for the ABI 31XX, 3730 and 500 Genetic Analyzers, or the 5-Color Dye Set –Single Capillary for the ABI 310 Genetic Analyzer (BI Cat# 20-911-00).
- Size Standard: 3ZOMY size standard (BI Cat# 20-920-00), or GeneScan™ 600 LIZ® Size Standard (Life Technologies Cat# 4366589).

2.3 Storage and Handling Requirements

a. Store all components below -18°C.

b. The activated reaction mixes (prepared by adding the 3ZOMY Chromosome Mix to the PCR) may be stored at +2° to +8°C for at least 7 days, and at below -18°C for at least 90 days.

c. Avoid repeated freeze-thawing.

d. Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.

e. Do not mix reagents from different kit lot numbers.

2.4 Symbols used on Labels

	Lot or batch number		Expiry date		Manufacturer
	Number of tests		In vitro diagnostic device		
	Store below temperature shown		Catalogue number		

3. Before You Begin

3.1 Precautions

a. The laboratories should process their own validation studies and quality control (QC) measures. Internal QC samples of known genotypes should be processed in each assay to assess the validity of the procedure.

b. Work under sterile conditions to avoid contamination, e.g., bacterial.

c. Do not pool reagents from different lots or from different vials of the same lot.

d. Do not use the kit after its expiry date.

e. Each test is for single-use only.

f. Do not use opened or damaged kit reagent vials.

g. Work flow in the laboratory should proceed in a unidirectional manner:

- Begin in the reagent preparation area, move to the DNA extraction area, then to the amplification area, and finally to the detection area.
- Pre-amplification activities should begin with reagent preparation and proceed to DNA extraction.
- Reagent preparation activities and DNA extraction activities should be performed in separate areas.
- Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas.
- Gloves should be worn in each area and should be changed before leaving that area.
- Equipment and supplies used for reagent preparation should not be used for DNA extraction activities, for pipetting, or processing amplified DNA or other sources of target DNA.
- Amplification and detection supplies and equipment should remain in the amplification and detection area at all times.

h. Handling of kit components and samples, their use, storage, and disposal should be in accordance with the procedures defined by national biohazard safety guidelines or regulations.

i. Wear powder-free disposable gloves, laboratory coats, and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

3.2 Spectral Calibration

Proper generation of a matrix file is critical for evaluating fluorescent labeled multiplex systems with ABI Genetic Analyzers. A matrix must be generated for each individual instrument.

The Kit has been calibrated with the following dye sets:

- The 5-Color Dye Set (BI Cat# 20-910-00), required for spectral calibration on ABI 31XX, 3500, and 3730 Genetic Analyzers.
- The 5-Color Dye Set –Single Capillary (BI Cat# 20-911-00), for ABI 310 Genetic Analyzer.

For protocols and additional information about spectral calibration, see the 3ZOMY Spectral Calibration protocol that can be obtained by request from BI's technical support team.

4. PCR: Sample Preparation and Amplification Setup

4.1 DNA Extraction

The 3ZOMYKit is for use with human genomic DNA extracted from whole blood, amniotic fluid, and chorionic villus samples. The product is not intended nor tested to be used for non invasive procedures.

The 3ZOMY Kit has been validated using the QIAamp DNA Blood Mini Kit (QiagenCat#51104) for extraction of DNA from human whole blood and amniotic fluid (AF), and QIAamp DNA Mini Kit (QiagenCat#51304) for extraction of DNA from chorionic villus (CV) biopsies. It is recommended that alternative DNA extraction methods and sample materials using the 3ZOMY Kit is carefully evaluated prior to the results being used for diagnostic use.

4.2 Working Solution Preparation

4.2.1 First-time use:

If the complete process is performed in one day, the working solution should be prepared before preparing the samples. The reverse order is advisable only if the samples are prepared the day before amplification or earlier.

The working solution is prepared by adding the Chromosome PCR Mix (yellow/white/red caps) to the PCR Enzyme (orange cap).

- Ensure that the Chromosome PCR Mix is completely thawed before use.
- Centrifuge the Chromosome PCR Mix tube briefly to collect the contents. Do not vortex the tubes at this step.
- Add 500µl of Chromosome PCR Mix to one tube of PCR Enzyme.
- Carefully mix by pipetting several times from the bottom of the tube.
- Vortex the working solution and centrifuge briefly to collect the contents.
- Add 20µl of the working solution to separate PCR reaction tubes.
- Cap the reaction tubes and centrifuge briefly to collect the contents.
- Continue to step 4.3.

Unused working solution should be aliquoted into 20µl per tube and frozen.

4.2.2 Subsequent use:

- Completely thaw the 20µl working solution tubes to be used.
- Continue to step 4.3.

4.3 Amplification Setup

Under optimal PCR conditions and using the recommended analysis settings (see below), acceptable results are consistently obtained at genomic DNA concentrations of 1-10ηg / PCR reaction.

The 3ZOMY Kit has been validated using a total PCR reaction volume of 25µl. Any change to the reaction volume may impair the Kit's performance.

We recommend using the following controls in every run:

- Negative control (no DNA)
- 46 chromosomes from known origin(normal DNA)
- 47 chromosomes from known origin for Chromosome 21 trisomy

Addition of Sample

Samples and controls should be added in a dedicated area separated from the reagent preparation, amplification, and detection areas.

Each PCR reaction should be prepared as follows:

Components	Samples	Positive Control	Negative Control
3ZOMY Working Solution (from step 4.2.2)	20µl	20µl	20µl
DNA	1-5µl (1-10ng)	1-5µl(1-10ng)	None
H_2O	Up to 5µl	Up to 5µl	5µl
Final PCR Reaction Volume	25µl	25µl	25µl

Cap the tubes and centrifuge briefly to collect the contents.

4.4 Thermal Cycling Protocol

The 3ZOMY Kit has been optimized for the GeneAmp PCR System 9700 thermal cycler.

Turn on the thermal cycler at least 30 minutes prior to amplification.

For ABI GeneAmp® System 9700 set “ramp speed” to “9600 mode”.

For use of alternative thermal cyclers the following ramping rates must be applied: heating 0.8°C/sec., cooling 1.6°C/sec.

Program the thermal cycler for amplification as follows:

Step	Temp / Time / Cycle No.
1	95°C for 15 min
2	94°C for 30 sec
3	58°C for 90 sec
4	72°C for 90 sec
	For 26 cycles
5	72°C for 30 min

- Start amplification (duration approximately 3 hours).
- Following amplification, remove the tubes containing completed PCR amplification reaction from the thermal cycler and place into a suitable holder. Centrifuge briefly to collect the contents.
- Remove the caps carefully to avoid aerosol contamination.
- Do not bring amplified material into the pre-amplification areas. Amplified material should be restricted to the amplification and detection areas.

5. Capillary Electrophoresis: Sample Preparation and Instrument Setup

Refer to the respective ABI Genetic Analyzer User Manual for instructions on maintenance and handling. Prior to running the 3ZOMY Kit, the instrument must be spectrally calibrated to support detection of the 5-Color Dye Set. See Section 3.2 for details.

5.1 Sample Preparation

a. Prepare a loading solution by combining and mixing 2µl of the Size Standard (3ZOMY size standard (BI Cat# 20-920-00) or GeneScan™ 600 LIZ® Size Standard (Life Technologies Cat# 4366589)) with 100µl of Hi-Di™ Formamide (sufficient mix for 6 wells/tubes).

b. Vortex for 15 seconds.

c. Dispense 15µl of the loading solution into the required number of wells in a 96-well plate or into individual tubes (ABI310) to be placed on the Genetic Analyzer.

d. Add 1.5µl of the sample PCR product to the corresponding well/tube containing loading solution.

e. Seal the plate/tubes.

5.2 Running Parameters:

Instrument parameters	ABI 310	ABI 3100/3130	ABI 3500
Run parameters	**POP-4**	**POP-4/POP-7**	**POP-7**
Capillary Length	47 cm	36 cm	50 cm
Run Temperature	60°C	60°C	60°C
Injection Voltage	15 kV	1.5 kV	1.6 kV
Run Voltage	15 kV	15 kV	19.5 kV
Run Time	40 min	1500 s	1500 s

The amount of PCR product injected into the capillaries can be adjusted by increasing/decreasing the injection time and/or injection voltage.

5.3 Instrument Setup

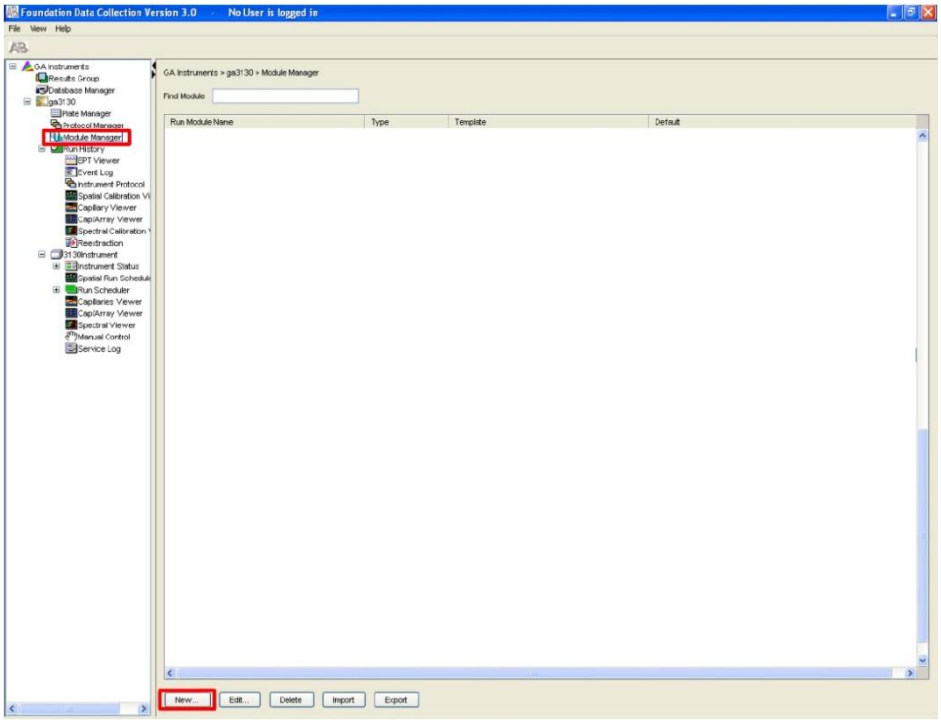
The following instructions are for an ABI 3130xl Genetic Analyzer with 3ZOMYKit. The dye set used is the 5-Color Dye Set 3010/3130/3500 (BI Cat# 20-910-00). However, the procedure is similar for the other instruments as well.

For further details, refer to the User Guide for the instrument used.

Before starting the electrophoresis for fragment analysis, the following settings need to be set-up in the instrument's Data Collection Software: Run Module, Instrument Protocol, and Plate.

5.3.1. Create a Run Module:

In the left navigation window select **Module Manager** and **New**.



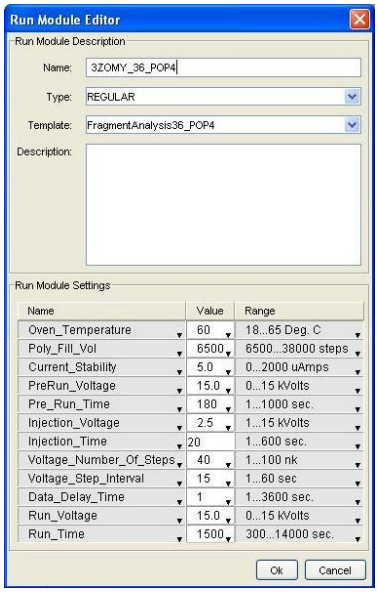
Fill out the Run Module Editor according to the Kit's instructions for use.

a. Name: Enter a name.

b. Type: Regular.

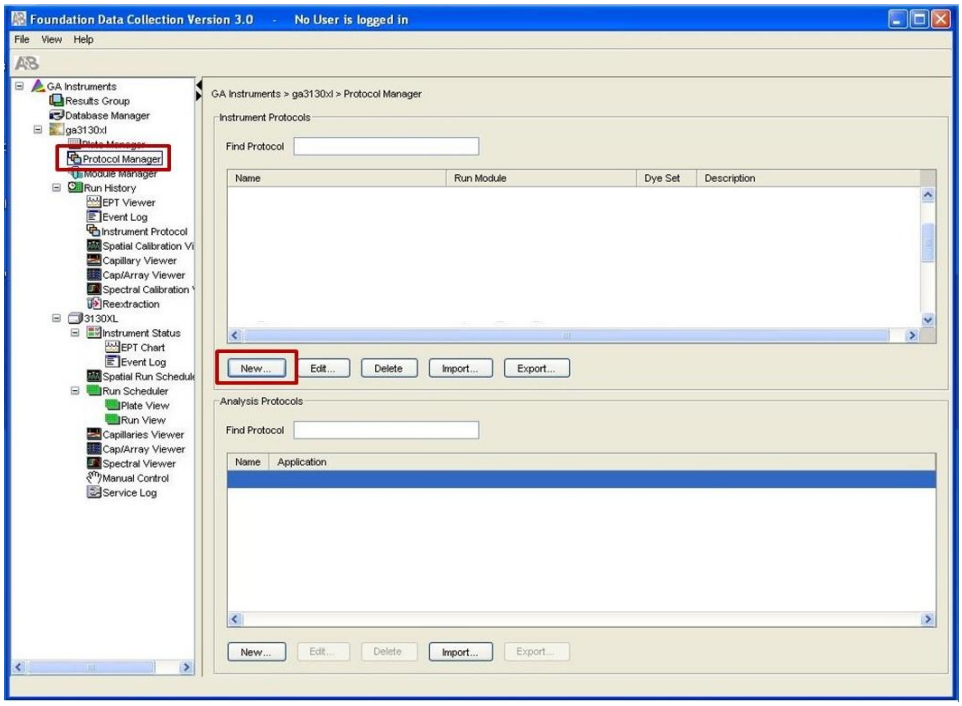
c. Template: FragmentAnalysis36_POP4 (default template for the capillary array and polymer used).

d. Specific Parameters: As specified in section 5.2.


Run Module Editor
Run Module Description
Name: 3ZOMY_36_POP4
Type: REGULAR
Template: FragmentAnalysis36_POP4
Description:
Run Module Settings
Name | Value | Range
Oven_Temperature | 60 | 18...65 Deg. C
Poly_Fill_Vol | 6500 | 6500...38000 steps
Current_Stability | 5.0 | 0...2000 uAmps
PreRun_Voltage | 15.0 | 0...15 kVolts
Pre_Run_Time | 180 | 1...1000 sec.
Injection_Voltage | 2.5 | 1...15 kVolts
Injection_Time | 20 | 1...600 sec.
Voltage_Number_Of_Steps | 40 | 1...100 nk
Voltage_Step_Interval | 15 | 1...60 sec
Data_Delay_Time | 1 | 1...3600 sec.
Run_Voltage | 15.0 | 0...15 kVolts
Run_Time | 1500 | 300...14000 sec.
Ok
Cancel

e. Click: OK.

5.3.2. Create an Instrument Protocol:



In the left navigation window select **Protocol Manager** and **New**.

a. Fill out the Protocol Editor

b. Name: Enter a name for the Run Module.

c. Type: Regular

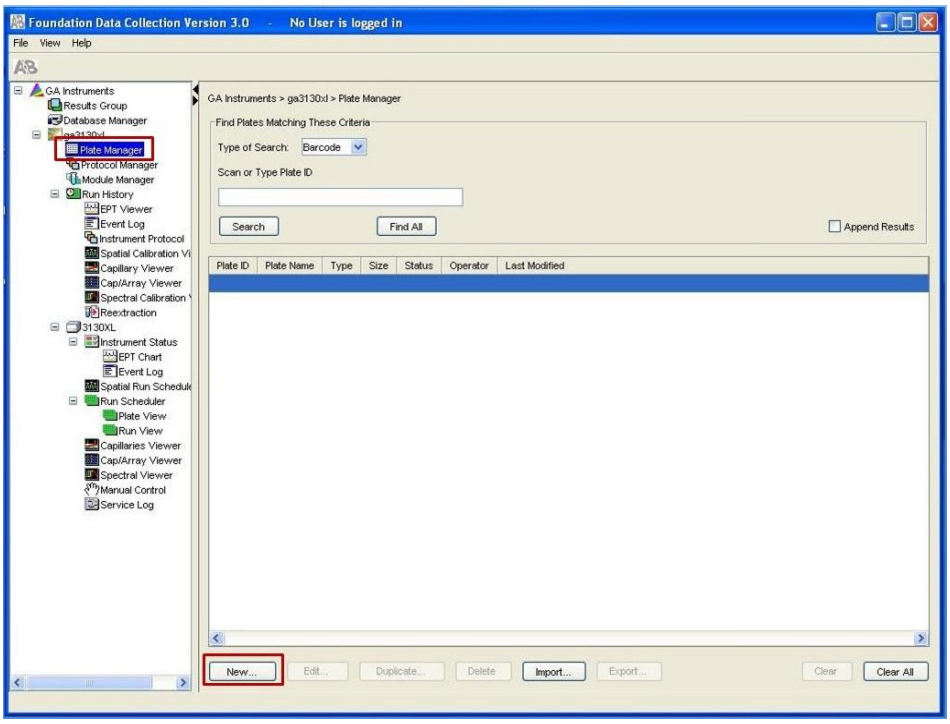
d. Run Module: Select the Run Module created in step 5.3.1:**3ZOMY_36_POP4**

e. Dye Set: 5-Color Dye Set.

f. Click: OK.

5.3.3. Set up a Plate for Run:

In the left navigation window select **Plate Manager** and **New**.



Fill out the new Plate Dialog:

a. Name: Enter a name for the plate.

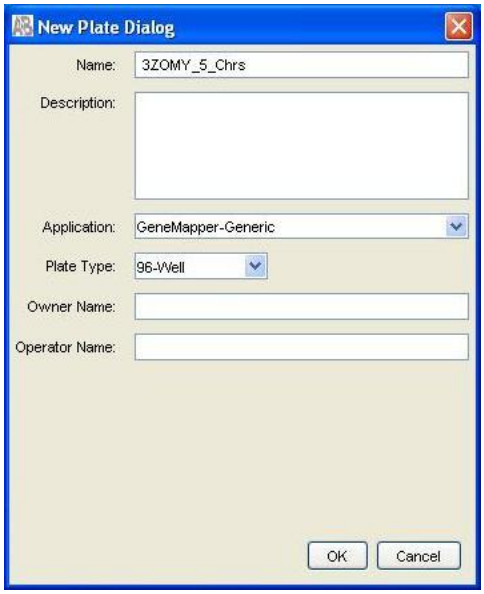
b. Application: GeneMapper-Generic

c. Plate Type: 96-Well

d. Owner Name: Enter the name of the owner

e. Operator Name: Enter the name of the operator.

f. Click: OK



Fill out the GeneMapper Plate Editor

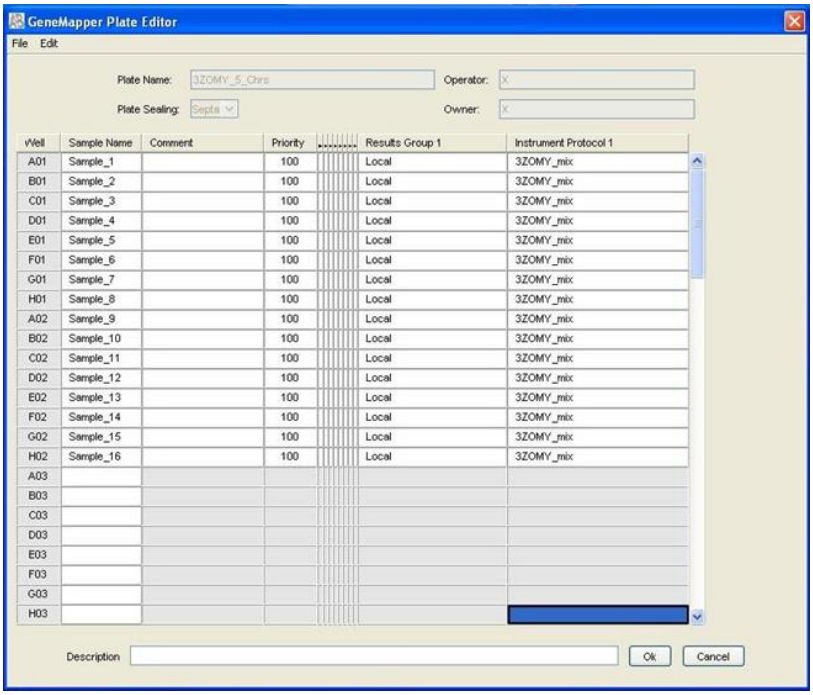
a. Sample Name: Enter the sample names.

b. Comment: Optional.

c. Results Group 1: Location where the raw data files (fsa files) will be saved.

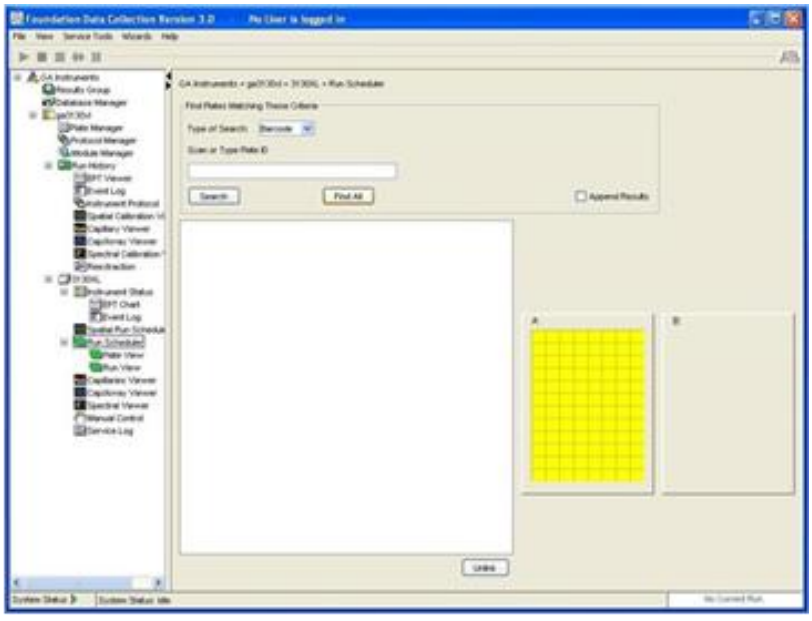
d. Instrument Protocol 1: Select the instrument protocol that you created in step 5.3.2.

e. Click: Ok

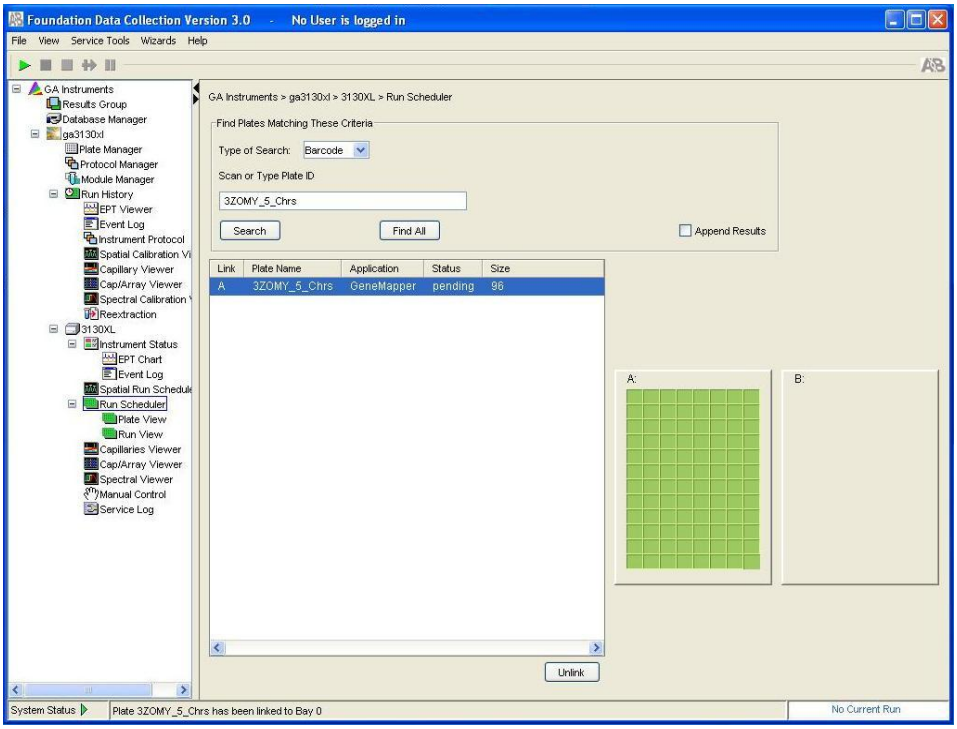


Well	Sample Name	Comment	Priority	Results Group 1	Instrument Protocol 1
A01	Sample_1		100	Local	3ZOMY_mix
B01	Sample_2		100	Local	3ZOMY_mix
C01	Sample_3		100	Local	3ZOMY_mix
D01	Sample_4		100	Local	3ZOMY_mix
E01	Sample_5		100	Local	3ZOMY_mix
F01	Sample_6		100	Local	3ZOMY_mix
G01	Sample_7		100	Local	3ZOMY_mix
H01	Sample_8		100	Local	3ZOMY_mix
A02	Sample_9		100	Local	3ZOMY_mix
B02	Sample_10		100	Local	3ZOMY_mix
C02	Sample_11		100	Local	3ZOMY_mix
D02	Sample_12		100	Local	3ZOMY_mix
E02	Sample_13		100	Local	3ZOMY_mix
F02	Sample_14		100	Local	3ZOMY_mix
G02	Sample_15		100	Local	3ZOMY_mix
H02	Sample_16		100	Local	3ZOMY_mix
A03					
B03					
C03					
D03					
E03					
F03					
G03					
H03					

a. In the left navigation window select **Run Scheduler** and **Find All**



b. Select the plate created in Step 5.3.3 (Status Pending). Link the plate by clicking on the yellow plate position indicator, which will turn green when linked. Start the run on the green arrow.



c. The Process Plates dialog box appears. Click OK to start the run

6. Data Analysis

One of the following software programs is required for data analysis: GeneMapper (Applied Biosystems) or GeneMarker (Softgenetics).

GeneMapper/GeneMarker settings for the 3ZOMY Kit and size marker must be imported before analysis of raw data. The latest versions can be obtained by request from BI's technical support team.

6.1 Importing New Panel and Bin Settings in the Panel Manager

a. Open the Panel Manager by selecting Tools and Panel Manager in the GeneMapper main menu or by clicking on the icon.

b. Import panels by clicking on the Panel Manager in the left navigation window. Panel Manager will now appear highlighted in blue.

c. Select File and Import Panels.

d. Navigate to and select the appropriate 3ZOMY Kit Panel file (.txt file) on your computer. Click Import.

e. The imported panel will now be displayed in the left navigation window.

f. Add a Bin Set to the imported panel by highlighting the panel in the left navigation window.

g. Select File and Import Bin Set.

h. Navigate to and select the appropriate 3ZOMY Kit Bin Set file (.txt file) on your computer. Click Import.

i. The imported Bin Set will appear in the Bin Set drop-down list. Click Apply and OK to confirm the imported panel and bin settings.

6.2 Importing GeneMapper Analysis Settings in the GeneMapper Manager

a. Open the GeneMapper Manager by selecting Tools and GeneMapper Manager in the GeneMapper main menu, or by clicking on the icon.

b. In the GeneMapper Manager select the Analysis Methods tab, and then click Import.

c. Navigate to and select the appropriate 3ZOMY Kit Analysis Methods file (.xml file) on your computer. Click Import.

d. The imported Analysis Method will now be listed in the Analysis Methods tab.

e. Repeat the process, selecting the appropriate tab and importing the corresponding file for:

Plot Settings

Table Settings

Report Settings

Size Standards

f. Select "Done" to confirm the imported analysis settings.

Note: A matrix file (mtx file) needs to be generated or imported in the Matrices tab when analyzing raw data generated from capillary electrophoresis using the ABI 310 Genetic Analyzer.

6.3 Performing Data Analysis

a. Import the raw data files (fsa files) from the capillary electrophoresis by selecting File and Add Samples to Project, or by clicking on the icon.

b. Navigate to the desired run folder using the folder tree in the left navigation window. Double click or use the plus symbol to display all files contained in the folder. Highlight the complete folder or selected samples, and click on Add to List.

c. The added run folder will now appear in the Samples to Add window. Add the samples by clicking on Add.

d. Set the appropriate analysis settings (Analysis Method, Panel and Size Standard) for each sample, alternatively fill down the columns by selecting the settings for one sample, highlighting the desired number of samples, and typing Ctrl D keys to copy the settings. Note: The matrix has to be set when analyzing fsa files from an ABI 310 Genetic Analyzer.

e. Initiate the data analysis by selecting Analysis and Analyze or by clicking on the icon. Assign a project name when prompted.

6.4 Reviewing QF-PCR Data

a. Highlight the sample row for the sample(s) to be selected for analysis in the GeneMapper main window.

b. Display the sample plot by selecting Analysis and Display Plots, or by clicking on the icon.

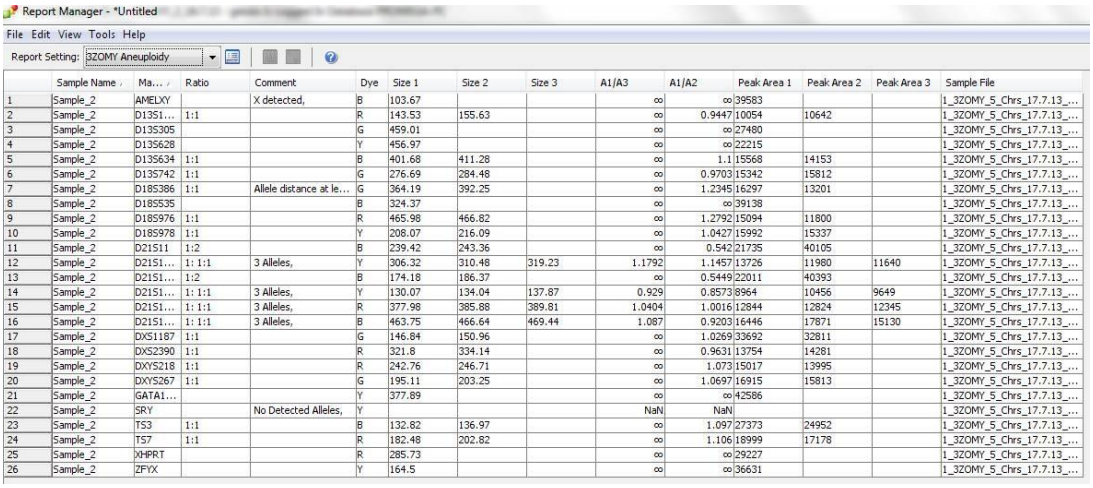
c. In the Samples Plot window, select the 3ZOMY Plot Setting from the drop-down menu.

d. To add or remove information shown in the peak labels, open the GeneMapper Manager, select the Plot Settings tab. Highlight the 3ZOMY Plot Setting, and then click Open.

e. Select the Labels tab. In the Show Labels box, select the information to be shown on the labels by using the drop down lists in the Label 1 to Label 4 options. To confirm the modified label format, click OK and Done. Changes in the Plot Settings will be directly applied in the Samples Plot without re-analyzing the samples.

6.5 Using the GeneMapper Report Format

The Report format can be used to facilitate the calculation of peak area ratios for the markers included in the analysis.


Report Manager - *Untitled

File Edit View Tools Help

Report Setting: 3ZOMY Aneuploidy

	Sample Name	Ma...	Ratio	Comment	Dye	Size 1	Size 2	Size 3	A1/A3	A1/A2	Peak Area 1	Peak Area 2	Peak Area 3	Sample File
1	Sample_2	AMELXY		X detected,	B	103.67			∞	∞	39583			1_3ZOMY_5_Chrs_17.7.13_...
2	Sample_2	D13S1...	1:1		R	143.53	155.63		∞	0.9447	10054	10642		1_3ZOMY_5_Chrs_17.7.13_...
3	Sample_2	D13S305			G	459.01			∞	∞	27480			1_3ZOMY_5_Chrs_17.7.13_...
4	Sample_2	D13S628			Y	456.97			∞	∞	22215			1_3ZOMY_5_Chrs_17.7.13_...
5	Sample_2	D13S634	1:1		B	401.68	411.28		∞	1.1	15568	14153		1_3ZOMY_5_Chrs_17.7.13_...
6	Sample_2	D13S742	1:1		G	276.69	284.48		∞	0.9703	15342	15812		1_3ZOMY_5_Chrs_17.7.13_...
7	Sample_2	D18S386	1:1	Allele distance at le...	G	364.19	392.25		∞	1.2345	16297	13201		1_3ZOMY_5_Chrs_17.7.13_...
8	Sample_2	D18S535			B	324.37			∞	∞	39138			1_3ZOMY_5_Chrs_17.7.13_...
9	Sample_2	D18S976	1:1		R	465.98	466.82		∞	1.2792	15094	11800		1_3ZOMY_5_Chrs_17.7.13_...
10	Sample_2	D18S978	1:1		Y	208.07	216.09		∞	1.0427	15992	15337		1_3ZOMY_5_Chrs_17.7.13_...
11	Sample_2	D21S11	1:2		B	239.42	243.36		∞	0.542	21735	40105		1_3ZOMY_5_Chrs_17.7.13_...
12	Sample_2	D21S1...	1: 1:1	3 Alleles,	Y	306.32	310.48	319.23	1.1792	1.1457	13726	11980	11640	1_3ZOMY_5_Chrs_17.7.13_...
13	Sample_2	D21S1...	1:2		B	174.18	186.37		∞	0.5449	22011	40393		1_3ZOMY_5_Chrs_17.7.13_...
14	Sample_2	D21S1...	1: 1:1	3 Alleles,	Y	130.07	134.04	137.87	0.929	0.8573	8964	10456	9649	1_3ZOMY_5_Chrs_17.7.13_...
15	Sample_2	D21S1...	1: 1:1	3 Alleles,	R	377.98	385.88	389.81	1.0404	1.0016	12844	12824	12345	1_3ZOMY_5_Chrs_17.7.13_...
16	Sample_2	D21S1...	1: 1:1	3 Alleles,	B	463.75	466.64	469.44	1.087	0.9203	16446	17871	15130	1_3ZOMY_5_Chrs_17.7.13_...
17	Sample_2	DXS1187	1:1		G	146.84	150.96		∞	1.0269	33692	32811		1_3ZOMY_5_Chrs_17.7.13_...
18	Sample_2	DXS2390	1:1		R	321.8	334.14		∞	0.9631	13754	14281		1_3ZOMY_5_Chrs_17.7.13_...
19	Sample_2	DXYS218	1:1		R	242.76	246.71		∞	1.073	15017	13995		1_3ZOMY_5_Chrs_17.7.13_...
20	Sample_2	DXYS267	1:1		G	195.11	203.25		∞	1.0697	16915	15813		1_3ZOMY_5_Chrs_17.7.13_...
21	Sample_2	GATA1...			Y	377.89			∞	∞	42586			1_3ZOMY_5_Chrs_17.7.13_...
22	Sample_2	SRY		No Detected Alleles,	Y				NaN	NaN				1_3ZOMY_5_Chrs_17.7.13_...
23	Sample_2	TS3	1:1		B	132.82	136.97		∞	1.097	27373	24952		1_3ZOMY_5_Chrs_17.7.13_...
24	Sample_2	TS7	1:1		R	182.48	202.82		∞	1.106	18999	17178		1_3ZOMY_5_Chrs_17.7.13_...
25	Sample_2	XHPRT			R	285.73			∞	∞	29227			1_3ZOMY_5_Chrs_17.7.13_...
26	Sample_2	ZFYX			Y	164.5			∞	∞	36631			1_3ZOMY_5_Chrs_17.7.13_...

The 3ZOMY Report Setting is included in the product settings folders. Use the 3ZOMY Table Setting with the corresponding 3ZOMY Report Setting.

Report and Table Settings are imported into GeneMapper through the GeneMapper Manager tool.

a. Select the appropriate Table Setting from the Table Setting drop-down menu in the GeneMapper main window.

b. Generate a report by highlighting the Sample file(s) to be analyzed, and then selecting Analysis and Report Manager, or by clicking on the Report Manager icon .

Or

In the Report Manager window select the appropriate Report Setting from the Report Setting drop-down menu.

c. To review the report data in a Samples Plot: Highlight a marker to be reviewed and then click on the icon.

To edit data in the Samples Plot window: right click on the marker and select the appropriate function in the menu box that appears. Any changes made will appear immediately in the Report.

6.6 Modifying the Analysis Method Setting

Depending on the ABI Genetic Analyzer used, it might be necessary to modify the Minimum Peak Height in the Analysis Method.

a. In the Analysis Method provided for the kit, the default Minimum Peak Height is applicable for instruments with a lower detection scale, i.e., ABI 310, ABI 3100, and ABI3130 (maximum detection 8000 RFU). When using ABI 3500 or ABI 3730 Genetic Analyzers (maximum detection at 32000 RFU), the Minimum Peak Height needs to be adjusted.

b. Open the GeneMapper Manager by selecting Tools and GeneMapper Manager in the GeneMapper main menu or by clicking on the icon.

Or

In the GeneMapper Manager, select the Analysis Methods tab. Highlight the desired Analysis Method, and then click Open.

c. Select the Peak Detector tab. The user specified (RFU) values of 100 for the blue, green, yellow, and red, and 50 for the orange, are applicable for the ABI 310, 3100, 3130 Genetic Analyzers.

If using an ABI 3500 or 3730 Genetic Analyzer, values of 500 for the blue, green, yellow, and red, and 200 for the orange, are applicable.

d. Click OK and Done after entering the user specified (RFU) values.

6.7 Missing Allele Calls – Analysis Method and Bin Set Connection Lost

Occasionally the Analysis Method looses its connection with the Bin Set. This is the likely explanation when the allele calls and bins cannot be displayed. Instead, question marks are displayed in the peak labels. Follow the instructions below to ensure that the Bin Set is connected to Analysis Method.

a. To connect the Bin Set to the Analysis Method, open the GeneMapper Manager by selecting Tools and GeneMapper Manager in the GeneMapper main menu, or by clicking on the icon . Or

In the GeneMapper Manager select the Analysis Methods tab.

b. Highlight the desired Analysis Method, and then click Open.

c. In the Analysis Method Editor, select the Allele tab. If the Bin Set has been lost, the Bin Set is set to none. Make sure that the correct Bin Set is added (in this example 3ZOMY).

d. Click OK and Done. If the Analysis Method has been modified, the samples need to be reanalyzed.

6.8 DISCLAIMER

RESULTS OBTAINED WITH ANY IVD KIT SHOULD ONLY BE EMPLOYED AND INTERPRETED WITHIN THE WHOLE CLINICAL PICTURE. BIOLOGICAL INDUSTRIES LTD. CANNOT BE CONSIDERED RESPONSIBLE FOR ANY CLINICAL DECISIONS TAKEN.

THIS KIT IS NOT INTENDED TO BE USED FOR THE RISK EVALUATION OF TRISOMY 21

7. Interpretation of Results

For interpretation of results, see Best Practice Guidelines for QF-PCR at **http://www.cmgs.org.**

1) At least one peak should be observed for each marker tested. The acceptable range for marker peaks analyzed on the 3130 Genetic Analyzer is between 50 and 6000 RFU, and for the 3500 Genetic Analyzer between 175 and 32000 RFU. Peak heights falling outside this range must not be analyzed.

2) The negative control should not show peaks above 50 RFU.

3) The positive control must show all the expected peaks.

4) To interpret a result as normal for a particular chromosome, at least two informative markers consistent with a normal genotype are required, with all other markers being uninformative.

5) To interpret a result as abnormal for a particular chromosome, at least two informative markers consistent with an abnormal genotype are required, with all other markers being uninformative.

6) *Normal allelic pattern is determined by:* Marker showing two peaks of similar height/area, and the peak ratio is classified as 1:1.

7) *Abnormal allelic pattern is determined by:*
   a) Marker showing two peaks of differing height/area and the peak ratio is classified as 2:1 or 1:2 (Trisomic Diallelic pattern).
   b) Marker showing three peaks of similar height/area and the peak ratio is classified as 1:1:1 (Trisomic Triallelic pattern).

8) The Kit includes three X-chromosome counting markers (TS2, TS3, and TS7) to be used to determine the number of X chromosomes when monosomy X is suspected.

9) *X-chromosome counting markers:*
The TS2, TS3, and TS7 markers are non-polymorphic X-chromosome counting markers that may be used to determine the number of X chromosomes when monosomy X is suspected. The X-chromosome counting markers define sequences present on the X chromosome and an autosomal chromosome that are amplified using identical primers. The amplified marker fragments are separated according to length, and the X chromosomal copy number is determined by fragment area ratio calculation.

In a normal female an X-chromosome counting marker area ratio of 1:1 is expected (Fig. 5).
In normal males and females with monosomy X a 2:1 ratio is expected (Fig. 6).

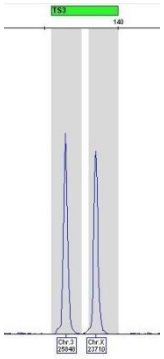


Fig. 5. Area ratio 1:1

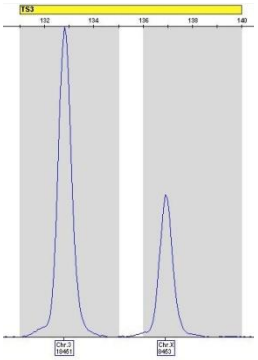


Fig.6. Area ratio 2:1

10) *Monosomy X pattern is determined by:*

a) All X and XY markers showing a homozygous allelic pattern.

b) The AMELY and SRY peaks are not detected.

c) Marker TS3 showing two peaks of differing height/area, and the peak ratio is classified as 2:1.

d) Marker TS7 showing two peaks of differing height/area, and the peak ratio is classified as 2:1.

11) *Non-Polymorphic Markers:*
The AMELXY and SRY markers amplify non-polymorphic sequences on the X (AMELX) and Y (AMELY and SRY) chromosomes, and can be used to determine the presence or absence of a Y chromosome. SRY will give a single peak in normal males.
AMELXY may be used to assess the relative number of X to Y chromosomes.

12) *Pseudo-Autosomal XY Markers:*

a) The DXYS267 and DXYS218 markers are polymorphic STR markers present on both the X and Y chromosomes.

b) The ZFY, ZFX marker is a non-polymorphic (non-STR) marker present on both the X and Y chromosomes.

c) These markers may be used to assess the total number of sex chromosomes when informative.

d) It is not possible to determine which allele represents the X or Y chromosome.

13) The use of the additional PCR mixes for chromosome 21 and sex chromosomes is recommended as back-up in case all the markers in the 5-Chromosome Mix are non-informative (homozygous), and can also be used individually for the respective aneuploidy diagnosis.

14) Analysis of heterozygous markers displaying two allele peaks is performed by calculating peak area ratios (Peak1/Peak2). Peak1 is the peak area of the shorter length fragment, and Peak2 is the peak area of the longer length fragment.

Ratio Criteria (RC) Interpretation:

Due to the occasional preferential amplification of the smaller allele, the ratio between fluorescent peaks may vary within limits shown in the following table:

Ratio	1:2	Inconclusive	1:1	Inconclusive	2:1
RC 1*	<0.65	0.65-0.74	0.75-1.44	1.45-1.75	>1.75
RC 2**	<0.65	0.65-0.74	0.75-1.54	1.55-1.75	>1.75

For markers displaying three allele peaks the ratio is always calculated starting with the area of the shortest length fragment (Peak1), i.e.,Peak1/Peak2, and Peak1/Peak3, respectively.

Ratio	Inconclusive	1:1:1	Inconclusive
RC 1*	<0.74	0.75-1.44	>1.45
RC 2**	<0.74	0.75-1.54	>1.55

*RC 1 is used when peak distance is <24bp

**RC 2 is used when peak distance is ≥24bp

Homozygous markers are considered uninformative.

15) Height Ratio

a) Height ratio may be calculated when an area ratio is classified as inconclusive. The same RC is applied as for area ratio calculation.

b) Allele ratios that fall between the normal and abnormal ranges are classed as inconclusive and must be re-analyzed.

c) If both normal and abnormal allelic patterns are obtained for a particular chromosome, it is recommended that follow-up studies be performed to identify the reason.

8. Troubleshooting

If heterozygous markers results fall in the inconclusive range or are not detected, it may be due to a number of factors such as:

8.1 Unusual conditions:

Symptoms	Description	Comments
Maternal cell contamination	Profiles demonstrate the presence of two genotypes (a second genotype is seen at all the markers).	Amplification of the maternal DNA should be done.
Chromosome mosaicism	Skewed allele ratios and/or minor third allele peak are detected on a chromosome-specific group of markers. 15-25% of mosaicism could be detected by QF-PCR.	Skewed and/or extra peaks should be investigated further.
Microvariants	STRs occasionally demonstrate incomplete repeat sequences. The interrupted repeat can be 1, 2 or 3 bases long. Microvariants are designated by the number of full repeats and the size of the incomplete repeat eg. A 9.3 allele indicates 9 full repeats and a 3 base microvariant.	
Primer site polymorphism	Partial or complete allele drop-out. If a complete drop-out is seen in a normal sample, the profile shows apparent homozygosity for an individual marker. In the case of a trisomic sample the profile may show apparent disomy. Partial drop-out is seen as an additional peak at a reduced height which can result in skewed, inconclusive or apparent 1:2/2:1 allele ratios.	1. In rare cases, amplification failure due to mutation of the primer site has been reported for the AMELY sequence. 2. In rare cases, ratio skewing due to mutation of the primer site has been reported for the TS1 marker sequences.
Somatic microsatellite mutation	SMM is detected as a novel allele at a single locus and is probably caused by a mitotic replication error. It usually involves 1-2 repeats increase or decrease in the repeat unit. Can be identified in a profile when present in a subpopulation of cells (mosaic). SMMs are more frequent in CVS than in AF.	
Copy number variation (CNV)		Have been reported for markers D13S742, D13S634, D13S628, D13S305, D18S978, D18S535, D18S386, D21S11, D21S1411, D21S1437, XHPRT, DXYS218, AMELXY, ZFYX, and TS7.

8.2 PCR Artefacts:

Symptoms	Description	Comments
Stutter peaks	Are detected as extra peaks that are one repeat smaller than the actual STR allele. The stutter peak area is typically less than 15% of the corresponding STR peak area.	Stutter peaks may be included in the ratio calculation.
(-) A peaks	Are detected as extra peaks that are one base smaller than the full length (+A peak) PCR product due to incomplete addition of the 3' A residue.	(-) A peaks may be included in the ratio calculation.
Large difference in size between two alleles	PCR is a competitive reaction. Where alleles are separated by a large number of repeats, the shorter allele can be preferentially amplified, leading to an imbalance between the two alleles.	

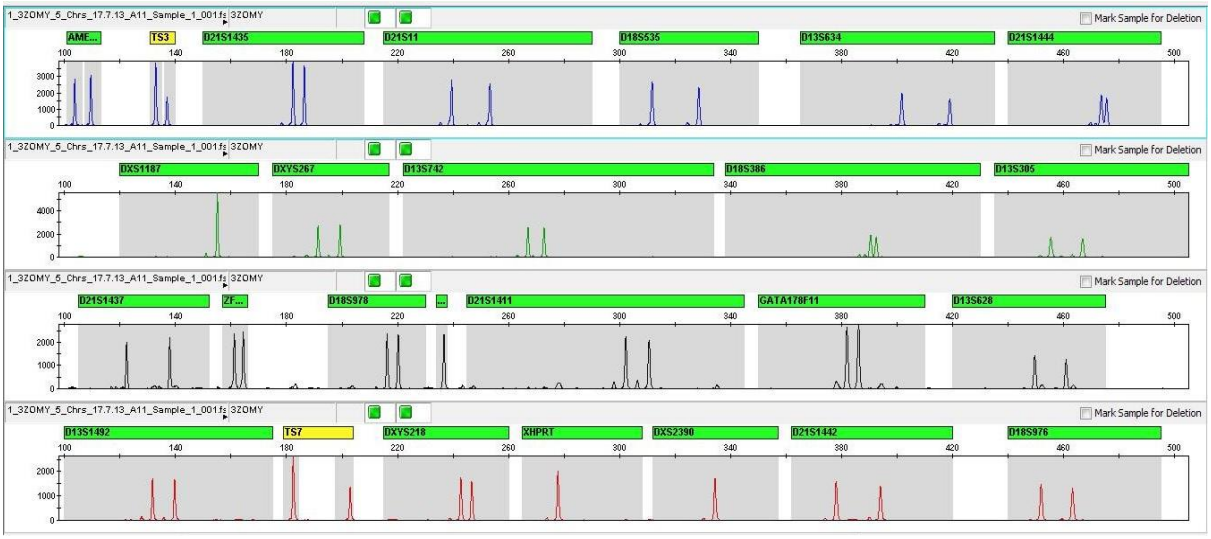
8.3 Electrophoretic Artifacts:

Symptoms	Description	Comments
Extra peaks visible in one or all channels	DNA concentration is too high.	Excessive amount of DNA on amplification.
Low signal – Missing peaks in one or all channels	DNA concentration is too low. Reduced amount of DNA template can result in preferential amplification of one allele resulting in skewing and/or uneven amplification of markers.	Insufficient template on amplification. If sufficient sample is available, it can be re-extracted and re-suspended in a lower volume.
Larger loci showing much more product than smaller loci.	DNA is degraded or of poor quality	
Pull-up or bleedthrough between dye channels	Can occur when peak heights are too high, or if a poor or incorrect matrix has been applied to the samples.	Pull-up peaks should be excluded from the analysis.
Dye blobs may appear in the sample analysis range	Dye blobs appear as broad, undefined peaks of a single color and tend to occur relatively early in the data.	Dye blobs should not be included in the analysis.
Failed analysis due to low sizing quality	Low sizing quality might be caused by insufficient peak height of the size standard fragments or incorrect identification of the fragments by the software due to sample overloading, spectral overlap or pre-terminated runs.	The size standard can be corrected manually in the **Size Match Editor**, or re-run the sample with a new Formamide-size standard mix.
Electropherogram data is of poor quality		Data should not be interpreted. The PCR product may be re-injected and re-analyzed.
Unexpected electrophoretic spikes	Minor voltage changes or urea crystals passing the laser.	Re-inject samples for confirmation.

9. Analysis Examples

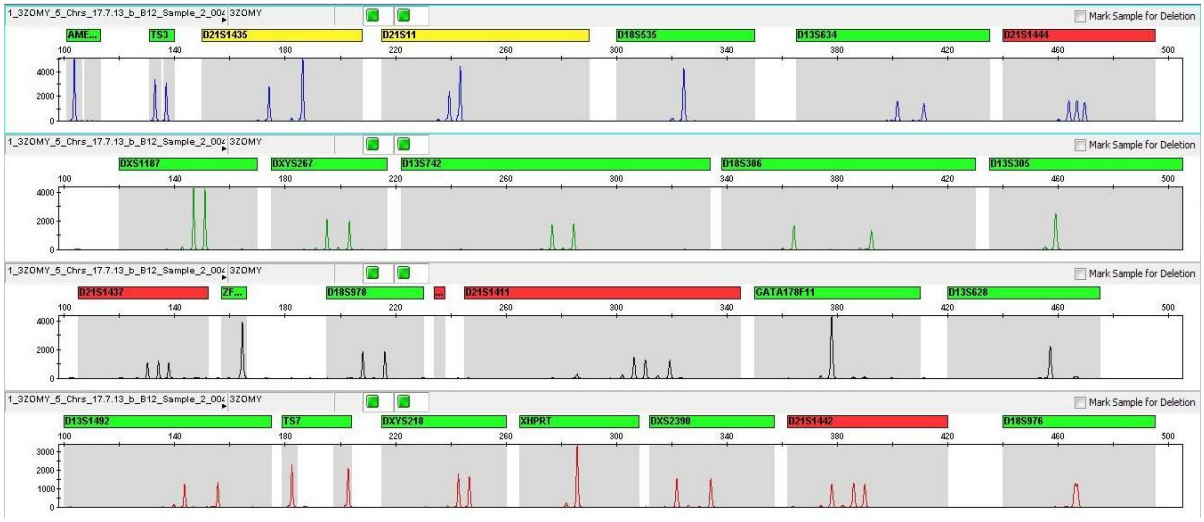
Normal Male (46, XY) Sample (5 Chromosome PCR Mix)

- All informative autosomal STR markers demonstrate a normal 1:1 marker ratio.
- The presence of AMELY and SRY is consistent with male gender.
- The X-chromosome counting markers (TS3 and TS7) demonstrate a 2:1 marker ratio, and all the X-chromosome STR markers are uninformative, in line with the expected dosage of X chromosomes in a normal male.
- The 1:1 marker ratio of the informative pseudo-autosomal XY chromosome STR markers (DXYS267 and DXYS218) and the non-polymorphic XY markers (AMELXY and ZFYX) confirm a normal male sex chromosomal dosage.



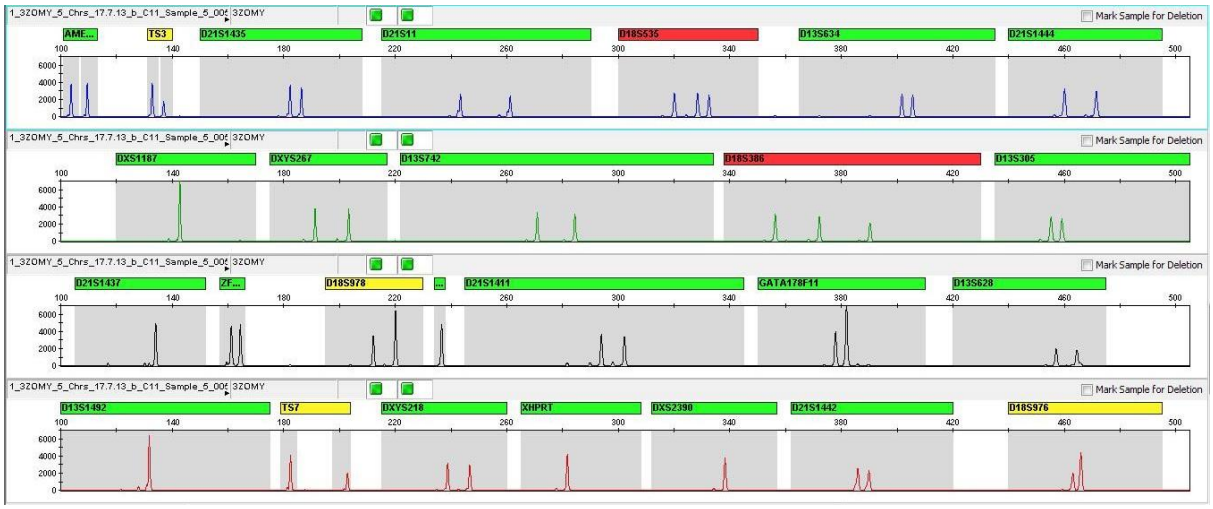
Down Syndrome, Trisomy 21 (47, XX, +21) Sample (5 Chromosome PCR Mix)

All informative autosomal STR markers on chromosome 21 demonstrate abnormal 1:2, 2:1, or 1:1:1 marker ratios consistent with trisomy 21.



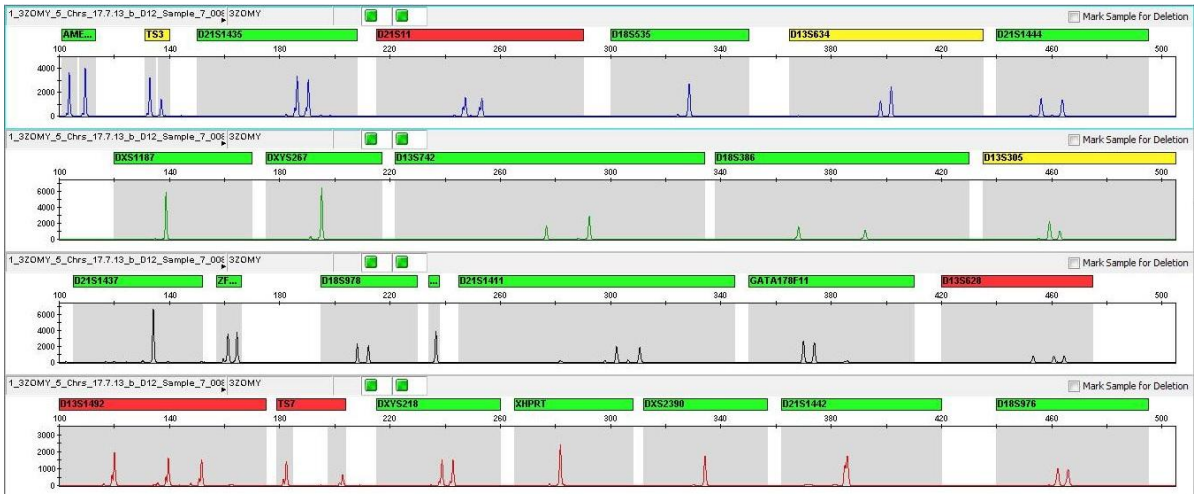
Edwards Syndrome, Trisomy 18 (47, XY, +18) Sample (5 Chromosome PCR Mix)

All informative autosomal STR markers on chromosome 18 demonstrate abnormal 1:2, 2:1, or 1:1:1 marker ratios consistent with trisomy 18.



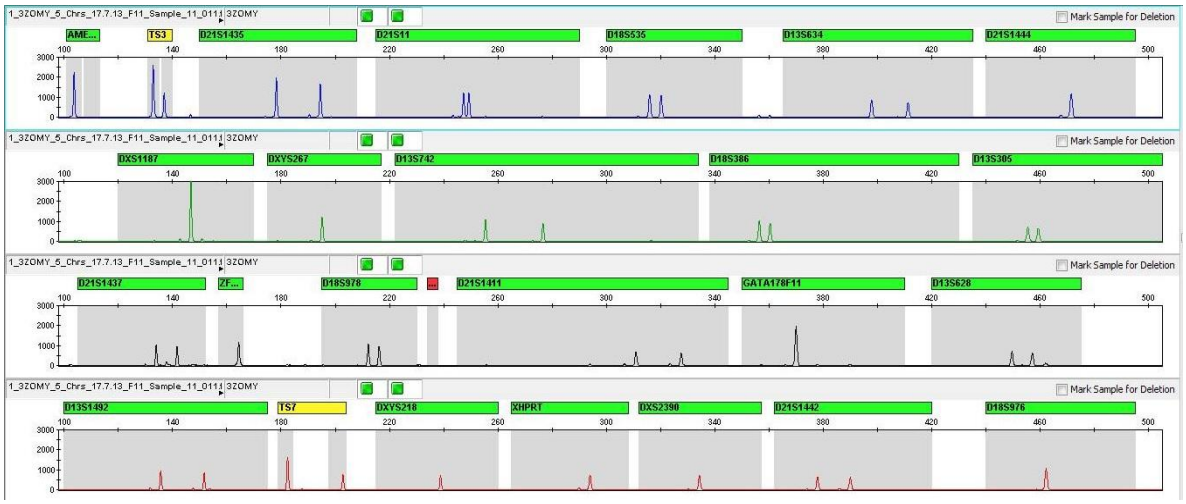
Patau Syndrome, Trisomy 13 (47, XY, +13) Sample (5 Chromosome PCR Mix)

All informative autosomal STR markers on chromosome 13 demonstrate abnormal 1:2, 2:1, or 1:1:1 marker ratios consistent with trisomy 13.



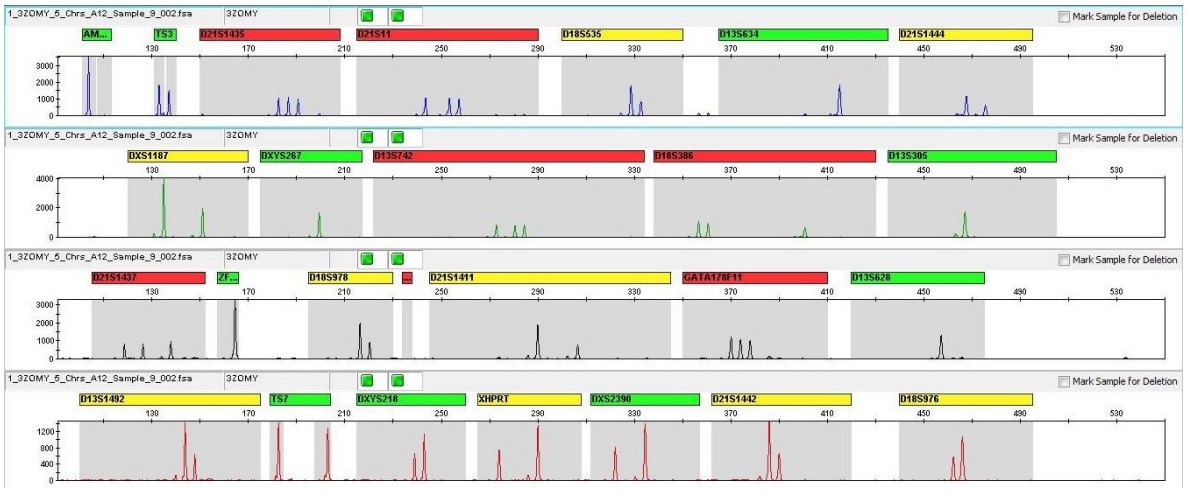
Turner Syndrome (45, X) Sample (5 Chromosome PCR Mix)

- All informative autosomal STR markers demonstrate a normal 1:1 marker ratio.
- The presence of AMELX and the absence of AMELY and SRY confirm female gender.
- The X-chromosome counting markers (TS3 and TS7) demonstrate an abnormal female 2:1 marker ratio consistent with the dosage of a single X chromosome.
- All X and XY STR markers are uninformative (DXS1187, DXYS267, DXYS218, XHPRT, DXS2390), consistent with the dosage of a single X chromosome.



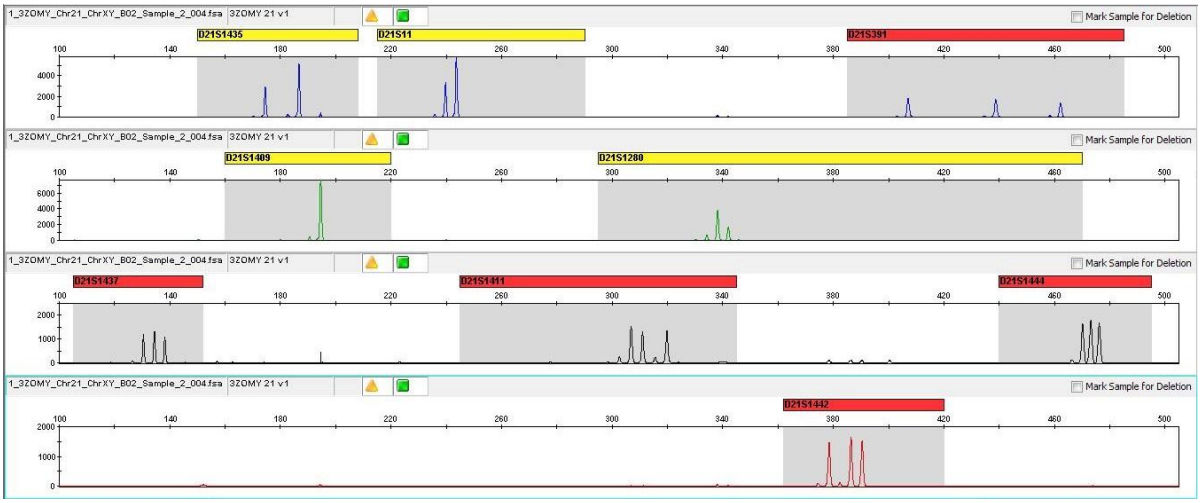
Triploidy Sample (5 Chromosome PCR Mix)

All informative STR markers on chromosomes 13, 18, 21, X, and Y demonstrate abnormal 1:2, 2:1, or 1:1:1 marker ratios consistent with triploidy.



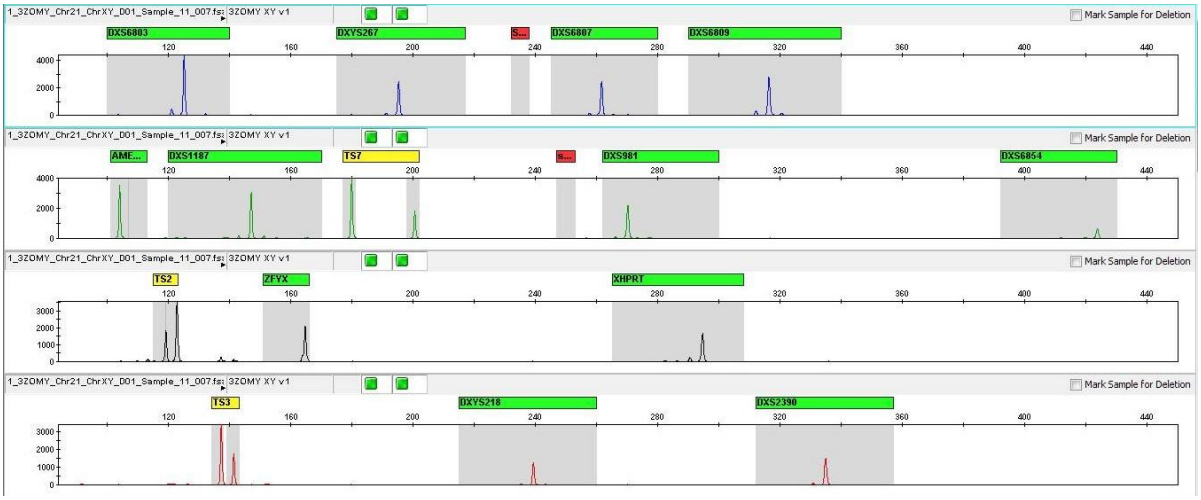
Down syndrome, Trisomy 21 (47, XX, +21) Sample (Chromosome 21 PCR Mix)

All STR markers demonstrate abnormal 1:2, 2:1, or 1:1:1 marker ratios consistent with trisomy 21.



Turner Syndrome (45, X) Sample (Sex Chromosome PCR Mix)

- The presence of AMELX and the absence of AMELY and SRY confirm female gender.
- The X-chromosome counting markers (TS3 and TS7) demonstrate an abnormal female 2:1 marker ratio consistent with the dosage of a single X chromosome.
- All X and XY STR markers are uninformative (DXS1187, DXYS267, DXYS218, XHPRT, DXS2390), consistent with the dosage of a single X chromosome.



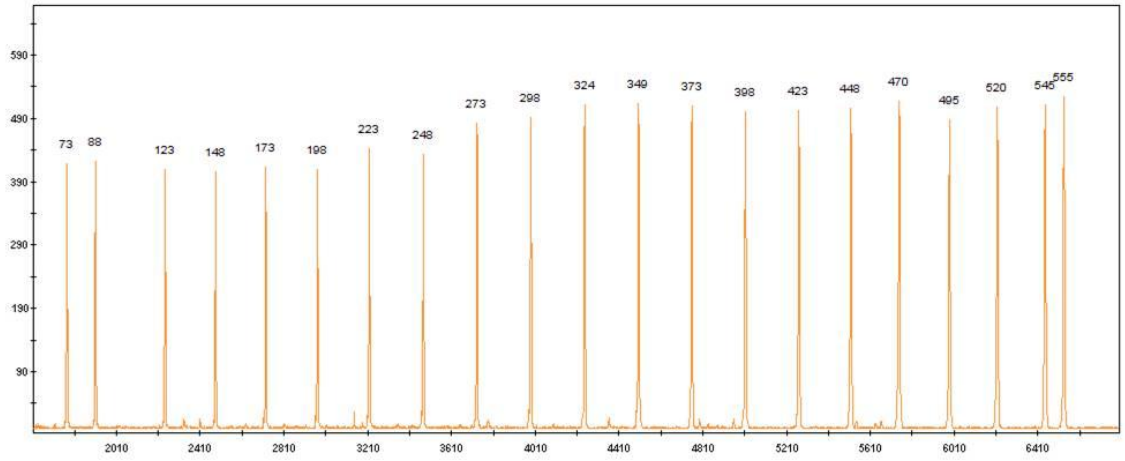
10. References

Professional guidelines for clinical cytogenetics and clinical molecular genetics .QF-PCR for the diagnosis of aneuploidy best practice guidelines (2012), v3.01, January 2012.

11. Appendixes

11.1 The3ZOMY size standard (BI Cat# 20-920-00)

The 3ZOMY size standard contains 21 DNA fragments of 73, 88, 123, 148, 173, 198, 223, 248, 273, 298, 324, 349, 373, 398, 423, 448, 470, 495, 520, 545, and 555 bases in length. It is detected as a fourth color in the presence of 3ZOMY amplified DNA in the orange channel.



11.2 Advantages of Using the Loci in the 3ZOMY Kit

3ZOMY QF-PCR has the major advantage of providing rapid, accurate, and cost-effective analysis for the diagnosis or exclusion of aneuploidy in chromosomes 13, 18, 21, X, or Y.

- ➔ The complete procedure from sample to results takes less than five hours, thus enabling your laboratory to provide results in just one day.
- ➔ Requires small amounts of genomic DNA.
- ➔ The 3ZOMY Kit's excellent assay performance and large number of highly informative markers significantly reduce the number of re-runs. In addition, a single PCR mix and detection reduces the risk of sample mix-up.

- The kit includes:
  - ★ 16 different somatic markers for chromosomes 21, 18, and 13, and 10 different sex chromosome markers to perform initial aneuploidy diagnosis.
  - ★ Two additional chromosome-specific marker sets for chromosomes 21 and XY to confirm the initial diagnosis or to be used individually for the trisomy of chromosome 21 and sex chromosome aneuploidy.
  - ★ Three unique X-chromosome counting markers for reliable detection of Turner syndrome.
- 3ZOMY can be used for maternal cell contamination detection.
- 3ZOMY is a CE-IVD certified kit.
- BI provides a complete technical support service.

11.3 Contact Information

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K
Pitampura, Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 8111, 8222
Mobile: +91-9810521400
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com